Physical exercise prevents the development of type 2 diabetes mellitus in *Psammomys obesus*

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**Heled, Yuval, Yair Shapiro, Yoav Shani, Dani S. Moran, Lea Langzam, Sanford R. Sampson, and Joseph Meyerovitch.** Physical exercise prevents the development of type 2 diabetes mellitus in *Psammomys obesus*. *Am J Physiol Endocrinol Metab* 282: E370–E375, 2002.—We hypothesized that exercise training might prevent diabetes mellitus in *Psammomys obesus*. Animals were assigned to three groups: high-energy diet (CH), high-energy diet and exercise (EH), and low-energy diet (CL). The EH group ran on a treadmill 5 days/wk, twice a day. After 4 wk, 99% of the CH group were diabetic compared with only 20% of the EH group. There was no difference in weight gain among the groups. Both EH and CH groups were hyperinsulinemic. Epididymal fat (% of body weight) was higher in the CH group than in either the EH and or the CL group. Protein kinase C (PKC)-δ activity and serine phosphorylation were higher in the EH group. No differences were found in tyrosine phosphorylation of the insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase among the groups. We demonstrate for the first time that exercise training effectively prevents the progression of diabetes mellitus type 2 in *Psammomys obesus*. PKC-δ may be involved in the adaptive effects of exercise in skeletal muscles that lead to the prevention of type 2 diabetes mellitus.

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**Type 2 diabetes mellitus** is the most common metabolic disease in the world (12). It is associated with a number of complications, such as nephropathy, retinopathy, arteriosclerotic heart disease, and peripheral neuropathy, which most often result from the prolonged exposure to hyperglycemia (13). Physical exercise is known to improve glucose uptake through mechanisms unrelated to insulin signaling (9), as well as by improving insulin sensitivity (1, 28). Nevertheless, knowledge of the biochemical mechanisms underlying these phenomena remains incomplete, although there is a consensus regarding the role of the GLUT-4 glucose transporter in the improvement of glucose uptake after acute (11, 28) and chronic (23, 24) exercise. Furthermore, except from indirect, retrospective epidemiological studies (17, 22), the preventive effect of exercise training on the development of insulin resistance and type 2 diabetes mellitus has not been extensively examined. Prospective studies assessing the physiological and biochemical effects of physical training in human populations are complicated. Therefore, a suitable animal model may provide a unique research tool for such studies.

*Psammomys obesus* (sand rat) is an animal model of nutritionally induced insulin resistance and type 2 diabetes mellitus (14, 25). When transferred to a high-energy laboratory diet, it develops type 2 diabetes mellitus within several days to 2 wk. Four generally consecutive stages (*stages A, B, C,* and *D*) of progression to diabetes have been described: *A*, the original stage: normoglycemic and normoinsulinemic; *B*, hyperinsulinemic only, which is sufficient to maintain normoglycemia; *C*, hyperinsulinemia and hyperglycemia (blood glucose level >11.1 mmol/l); *D*, hyperglycemia and hypoinsulinemia, due to loss of β-cell insulin secretion capacity. *Stage D* is irreversible and, unless treated with insulin, the animals eventually die from severe ketoacidosis (14). Several possible mechanisms have been suggested that may be responsible for the inability of the animals to cope with high-energy nutrients: insufficient secretion of insulin, inappropriate response to insulin due to low sensitivity in peripheral tissues (10), and high metabolic efficiency in handling the nutrients taken in (14), which becomes deleterious as a result of a “thrifty” metabolism (25), part of which was suggested to involve protein kinase C (PKC)-ε (10). Studies regarding the effect of exercise on the development of diabetes in these animals have not been reported.

PKC is a family of serine-threonine kinases that plays an important regulatory role in a variety of biological phenomena (20), including insulin signaling (3, 4). The family is composed of a number of isoforms, which, according to structure and cofactor requirements for activation, can be grouped into three catego-
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determinations was taken at baseline and once a week from the tail vein, using the glucometer Elite (Bayer, Kyoto, Japan) in the EH group. Blood was taken 24 h after the preceding exercise. After 4 wk, the epididymal fat weight-to-body weight ratio (%) was calculated as an accepted indicator of obesity in Psammomys obesus (14). Serum insulin concentration was measured by RIA with the standard 18-h incubation double-antibody assay. Primary (guinea pig) and secondary (goat anti-guinea pig) antisera were from Linco Research (St. Charles, MO). Human insulin standard (Novo Nordisk, Bagsvaerd, Denmark) was used for Psammomys obesus insulin RIA; cross-reactivity and dilution linearity were previously determined (7). The minimum detectable concentration was 11 pmol/l; the routine intra-assay coefficient of variation (CV) was 4–6%, and the interassay CV was 6–10%. Serum triglyceride concentration was measured using a kit obtained from Sigma (St. Louis, MO; catalog number 536–10). To calculate the animals’ energy consumption, feed consumption in each cage of 5 animals was measured daily. The average feed consumed by one animal was then calculated by dividing this value by 5, and then multiplied by the suitable energy value (kJ/g) according to the type of feed.

Preparation of Muscle Tissue

Muscle tissue samples were washed with Ca\(^{2+}\)/Mg\(^{2+}\)-free PBS to remove excess blood cells and then were mechanically lysed in RIPA buffer (50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 10 mM NaF; 1% Triton X-100; 0.1% SDS; and 1% Na deoxycholate) containing a cocktail of protease inhibitors (20 \(\mu\)g/ml leupeptin; 10 \(\mu\)g/ml aprotonin; 0.1 mM phenylmethylsulfonyl fluoride; and 1 mM dithiothreitol) and phosphatase inhibitors (200 \(\mu\)M orthovanadate; 2 \(\mu\)g/ml pepstatin) from Sigma. After 30 s of homogenization in a Dounce glass homogenizer, the preparation was centrifuged at 20,000 \(\times\) g for 20 min at 4°C. The supernatant, containing all of the tissue’s proteins, was then stored at −70°C for later analysis.

Biochemical Measurements

The protein content in each sample was measured with the Bio-Rad protein assay according to the manufacturer’s instructions (Bio-Rad, Richmond, CA).

Immunoprecipitation. Twenty-five microliters of protein A/G Sepharose were added to 0.3 ml of the lysate, and the suspension was rotated continuously for 30 min at 4°C. The preparation was then centrifuged at 20,000 \(\times\) g for 10 min, and 30 \(\mu\)l of A/G Sepharose were added to the supernatant along with specific monoclonal or polyclonal antibodies to various antigens. The suspension was rotated overnight at 4°C. The suspension was then centrifuged at 20,000 \(\times\) g for 10 min at 4°C, and the pellet was washed twice as above with RIPA buffer. The beads were eluted with 25 \(\mu\)l of sample buffer (0.5 M Tris–HCl, pH 6.8; 10% SDS; 10% glycerol; 4% 2-mercaptoethanol; 0.05% bromophenol blue). The suspension was again centrifuged at 15,000 \(\times\) g (4°C for 10 min) and washed 4 times in TBST. Sample buffer was added, and the samples were boiled for 5 min and then subjected to SDS-PAGE.

Western blotting. Twenty to twenty-five micrograms of protein were electrophoresed through SDS-polyacrylamide gels (7.5 or 10%) and electrophoretically transferred onto Immobilon-P (Millipore, Bedford, MA) membranes. After transfer, the membranes were subjected to standard blocking and incubation procedures and were incubated with specific monoclonal or polyclonal antibodies to the various proteins. The membranes were washed 4 times for 15 min in TBST and

Materials and Methods

Animals

Forty-five male Psammomys obesus aged 6 wk (3 wk after weaning) from the Hebrew University, Hadassah Medical School Animal Farm were used in the present study. The animals were housed in suitable cages (5 animals in a cage) in a temperature (22–25°C)- and light (12:12-h light-dark cycle)-controlled room. The animals were randomly assigned to three groups of 15 animals each: EH, exercising animals consuming a high-energy diet (12.27 kJ/g; Weizmann Institute) that does not induce diabetes (25). Food and water were supplied ad libitum. All experimental procedures were authorized by the institutional animal care committee (protocol number 11/147/00).

Materials

Anti-phosphotyrosine and anti-phosphoserine were obtained from Upstate Biotechnology (Lake Placid, NY). Anti-insulin receptor (IR) and anti-phosphatidylinositol 3-kinase (PI 3-kinase) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). IR substrate-1 (IRS-1) was obtained from Transduction Laboratories (Lexington, KY). Anti-protein PKC-δ was obtained from Promega (Madison, WI).

Experimental Protocol

Three days before the experiment, the EH group was familiarized with the exercise training for 10–15 min/day. During the 4-wk protocol, the animals ran on a treadmill (Quinton Q55, Seattle WA; 2.25 km/h, 6% slope) 5 days a week for 15 min; the serum was kept at 70°C. Two milliliters of blood were then centrifuged at 20,000 \(\times\) g for 10 min, and the pellet was washed twice as above with RIPA buffer. The beads were eluted with 25 \(\mu\)l of sample buffer (0.5 M Tris–HCl, pH 6.8; 10% SDS; 10% glycerol; 4% 2-mercaptoethanol; 0.05% bromophenol blue). The suspension was again centrifuged at 15,000 \(\times\) g (4°C for 10 min) and washed 4 times in TBST. Sample buffer was added, and the samples were boiled for 5 min and then subjected to SDS-PAGE.

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then further incubated for 20 min at room temperature with horseradish peroxidase (HRP)-labeled secondary antibody (goat anti-rabbit or anti-mouse IgG) diluted 1:10,000 in blocking buffer. After 3 washes (1 x 15 min and 2 x 5 min) in TBST, the membranes were treated with enhanced chemiluminescence reagent for 1 min and then exposed on X-ray film (Kodak, Rochester, NY) for the required times (5–30 s) and developed.

Activity assay. PKC-δ activity was measured after immunoprecipitation with anti-PKC-δ antibody, as described in Immunoprecipitation. The lysates were prepared in RIPA buffer without NaF. Activity was measured with the use of the SignaTECT protein kinase C assay system (Promega). This kit contains all necessary cofactors and utilizes a highly specific biotinylated substrate (Neurogranin).

In preliminary experiments, we demonstrated that immunoprecipitation was specific for the PKC-δ isofrom. In other words, immunoblotting of the immunoprecipitated PKC-δ with specific antibodies to other isofroms did not reveal the presence of isofroms other than the one specifically immunoprecipitated.

Statistical Analysis

Results were analyzed using ANOVA followed by Tukey’s pairwise comparisons. Values where \( P < 0.05 \) were considered significant. Data are presented as means ± SE.

RESULTS

Physiological Profiles of CH, EH, and CL Groups During the Experiment

The physiological results after 4 wk are summarized in Table 1, and the dynamics of the glucose level changes during the 4 wk are presented in Fig. 1. After 4 wk, 14 (93%) of the CH animals became diabetic (average blood glucose level 21 ± 0.4 mmol/l) compared with only 3 (20%) of the EH animals (average blood glucose level 9.6 ± 1.9 mmol/l; \( P < 0.05 \)). It should be noted that the average blood glucose of the nondoniabetic animals in the EH group (n = 12) was 4.6 ± 0.3 mmol/l. Both groups had significantly higher blood glucose levels than the CL group, where no animal became diabetic (average blood glucose level 3.38 ± 0.38 mmol/l).

Table 1. Summary of physiological measurements of high-energy, high-energy exercise, and control low-energy groups of Psammomys obesus after 4 wk

<table>
<thead>
<tr>
<th></th>
<th>CH</th>
<th>EH</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>21 ± 0.4†‡</td>
<td>9.6 ± 1.9‡</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>4,900 ± 1,200†</td>
<td>4,600 ± 1,000†</td>
<td>300 ± 100</td>
</tr>
<tr>
<td>Triacylglycerol, mmol/l</td>
<td>1.4 ± 0.4‡</td>
<td>0.8 ± 0.1†‡</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Energy consumption in 3rd wk, kJ·animal(^{-1})·day(^{-1})</td>
<td>160.7 ± 9.9*</td>
<td>184 ± 9.9</td>
<td>169.5 ± 20</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>60 ± 7</td>
<td>71 ± 7</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Epididymal fat, % body wt</td>
<td>1.26 ± 0.29‡</td>
<td>0.7 ± 0.13</td>
<td>0.4 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15/group. Groups: CH, high-energy; EH, high-energy exercise; CL, control low-energy. †Significantly different from EH (\( P < 0.05 \)); ‡significantly different from CL (\( P < 0.01 \)); *significantly different from CL (\( P < 0.05 \)).

The physiological results as presented in Table 1 clearly point to the fact that the animals in the CH group were in the hyperinsulinemic-hyperglycemic stage (stage C) of the disease, whereas those in the EH group were maintained in the hyperinsulinemic-normoglycemic stage (stage B) of the disease.

Biochemical Results

We examined the possibility that changes in the tyrosine phosphorylation state of the major signaling proteins might account for the beneficial effects of exercise.

IR, IRS-1, and PI 3-kinase phosphorylation states. In initial experiments, we studied the phosphorylation state of upstream elements, such as the IR-β subunit, IRS-1, and PI 3-kinase. We did not detect any changes in tyrosine phosphorylation of IR, IRS-1, or PI 3-kinase among the different groups (results not shown).

PKC-δ activity. Among the downstream elements shown to be involved in regulation of glucose transport is the PKC family of serine-threonine kinases, in particular, PKC-δ (2). Accordingly, we measured the activity of the PKC-δ isoform. PKC-δ was immunoprecipitated from cell lysates of quadriceps muscles excised from animals in each group, and activity was measured as described in MATERIALS AND METHODS. As shown in Fig. 2, PKC-δ activity was significantly higher in the EH group compared with the CH and CL groups, with no difference between the latter two (440 ± 13 vs. 388 ± 20 and 356 ± 16 counts/min, respectively; \( P < 0.05 \)).
PKC-δ phosphorylation. Phosphorylation of tyrosine and serine residues has been demonstrated to be associated with the state of activity of PKC isoforms (18, 19). Anti-phosphotyrosine and anti-phosphoserine antibodies were used to probe immunoprecipitated PKC isoforms. Figure 3 shows that there was no difference in the level of PKC-δ tyrosine phosphorylation between the EH and CL group, but it was significantly higher in those groups than in the CH group. Serine phosphorylation of PKC-δ, similar to the activity results, was significantly higher in the EH group compared with the CH and CL groups, with no difference found between the latter two (Fig. 4).

DISCUSSION

The main purpose of the present study was to evaluate the effect of exercise training on preventing type 2 diabetes mellitus in the diabetic-prone animal model *Psammomys obesus* that rapidly develops the disease after consuming a high-energy diet. Our results showed that, in animals that underwent exercise training, the progression of the disease was prevented for ≥1 mo, whereas most of the control animals became severely diabetic after 1 wk. This prevention was not associated with a decreased weight gain in the EH group or hyperphagia in the CH group (Table 1). Referring to previous work showing that activated PKC-δ induces glucose transport in primary cultures of rat skeletal muscle via translocation of GLUT-4 (2), we provide evidence that muscle PKC-δ is activated by exercise and might be causally related to the prevention of diabetes in this animal model through yet unknown mechanisms. Except for one study on the OLETF (or Otsuka Long Evans Tokushima fatty) rat (26), which is a completely different diabetic-prone model, no prospective study has been reported in which exercise training prevented the development of type 2 diabetes in a genetically prone model. In addition, we think that *Psammomys obesus*, which has labile β-cells with transient insulin secretion capacity (25) (similar to humans) and more severe symptoms of the disease, is a more applicable model to treatment and prevention interventions and therefore more suitable for such studies.

It should be emphasized that, although exercise training prevented type 2 diabetes in the EH group, insulin resistance was only partially affected; this group was at the second stage (*stage B*) of the disease (14) as indicated by hyperinsulinemia. These results are not surprising, because *Psammomys obesus* are known to be insulin resistant even in their normal baseline (*stage A*) level (29). Interestingly, body weight changes were not significantly different among the groups. Similar results regarding only the CH and CL groups were found in another study (21), suggesting that the energy balance of all three groups was similar. It should also be noted that the EH group might gain weight by increasing their muscle mass due to the daily exercise training. As for the body fat content, although the present study’s results showed that the high-energy groups had higher epididymal body fat content, it was not enough to cause significant weight differences.

As for the biochemical results, we recently found that, in primary cultures of rat skeletal muscle, activated PKC-δ induces glucose transport via translocation of GLUT-4 (2), but PKC-δ activity in skeletal muscle after exercise was never studied before. Nevertheless, one study pointed to a direct effect of cardiac muscle contraction on PKC-δ activation (27). It was also hypothesized once that exercise-induced translocation of PKC (in general) and production of diacylglycerol were connected to the activation of glucose transport (5). We found that activity of PKC-δ was significantly higher in the EH group compared with the CH and the CL groups, with no difference between the latter two. The similar results in the two control groups, despite their different nutrition, might be due to the fact that the *Psammomys obesus* are insulin resistant even at their normal *stage A* (29). Therefore, we hypothesize that the PKC-δ activity remains low in the high-energy state, and the increase in activity of this isoform by physical exercise is partly responsible for the prevention of hyperglycemia.

Tyrosine phosphorylation results of PKC-δ were expected to be similar to those of activity (18). Indeed, tyrosine phosphorylation of skeletal muscle PKC-δ was higher in the EH group than in the CH group, but it
was not significantly different between the EH and CL groups. It might be suggested, therefore, that in this model, PKC-δ tyrosine phosphorylation does not necessarily represent the activation state, as was shown before by use of other models (8). Another possibility is that the diabetic state reduced PKC-δ tyrosine phosphorylation, whereas exercise training prevented this reduction. As for the PKC-δ serine phosphorylation, which represents the enzyme’s autophosphorylation and activation states (8), it was significantly higher in the EH group compared with the CH and CL groups. This suggests that autophosphorylation of PKC-δ is low at the baseline level in Psammomys obesus, remains low at the diabetic stage, and, similar to activity, increases after exercise training. It seems, therefore, that PKC-δ may be activated by chronic physical exercise. Our previous finding that activated PKC-δ induces glucose transport via translocation of GLUT-4 in skeletal muscle (2) may account for the association between exercise training and enhanced glucose uptake in skeletal muscle of Psammomys obesus.

We did not find any significant differences in tyrosine phosphorylation of the IR, IRS-1, or PI 3-kinase. These enzymes are known to be tyrosine phosphorylated and activated during hyperinsulinemia after acute (28) and chronic (6, 15, 16) physical exercise in higher levels than in the preexercise state, and, therefore, pronounce the higher insulin sensitivity due to exercise. The fact that the responses of these upstream elements to a hyperinsulinemic state in the CH and EH groups were not different and were also similar to the normoinsulinemic CL group further strengthens the fact that these animals are insulin resistant in their baseline stage (29). We also suggest, according to these results, that the prevention of the hyperglycemia and the progression of type 2 diabetes mellitus in the Psammomys obesus by the adaptive effects of exercise training occurred via distinct mechanisms that differ from conventional insulin signaling. Further studies that will measure translocation of GLUT-4, glucose uptake, and the response to insulin injection are required to further validate our results.

In conclusion, the present study demonstrates that exercise training is effective in preventing the progression of diabetes mellitus type 2 in Psammomys obesus. The increased PKC-δ activity due to exercise training appears as a possible adaptive regulatory-related mechanism that may enhance glucose uptake via GLUT-4 overexpression/translocation in skeletal muscle and, therefore, prevent the hyperglycemia in Psammomys obesus. The precise mechanisms proximal and distal to PKC-δ activation should be further studied. This mechanism is probably a part of other yet unknown adaptive mechanisms in the skeletal muscle through which exercise training prevents type 2 diabetes mellitus.

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